Partitioning of Rubisco and Chlorophyll in Abutilon theophrasti

Jeffrey H. Simonson

Plants likely balance their production of light-harvesting and carbon-fixing molecules in response to different environments. Low-light plants may increase chlorophyll production at the expense of carbon-fixing Rubisco; high-light plants may do the converse. We grew Abutilon theophrasti *in high/low light and high/low nitrogen. We measured [Chl] using photospectrometry and [Rubisco] using an antibody assay. The variation of both [Chl] and [Rubisco] within treatments was greater than the variation between treatments. This experiment does not support our partitioning hypothesis.*

Introduction

Plants exhibit phenotypic plasticity under various environmental conditions. To optimize performance, they must balance production of expensive molecules in response to their surroundings. Synthesis of both chlorophyll (Chl) and ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) involves significant nutrient and energy investments.

Plants may partition these molecules to fit light and nutrient availability. Under high light, they have the energy resources to produce more carbon-fixing Rubisco at the expense of chlorophyll production. Conversely, low-light plants must channel their resources into light-capturing chlorophyll and spend less on fixing carbon. This is what Besford (1986) found in a study using tomato. He measured a higher soluble protein to chlorophyll ratio in high-light plants than in low-light plants.

The purpose of this study is to show *Abutilon theophrasti* grown in high light has high [Rubisco] and low [Chl], while low-light plants have the opposite concentrations. Furthermore, since Rubisco has a high nitrogen content, nitrogen-stressed plants should have less Rubisco.

Methods

Plant Growth

We grew 10 *Abutilon theophrasti* in each of 4 different environments for a total of 40 plants. The treatments consisted of high/low light, and high/low nitrogen. Therefore, the four treatment groups were high light and high nitrogen (HH), high light and low nitrogen (HL), low light and high nitrogen (LH), and low light and low nitrogen (LL). All plants were fertilized with their appropriate solutions approximately every two days and given additional water as needed.

We harvested the plants after 26 days of growth. The harvest consisted of removing the sixth leaf down from the single apical meristem and punching out 1/4 inch diameter leaf discs. The discs were then stored at -60°C.

To measure relative growth rates we marked the petioles of the second leaf from the apical meristem. After 18 days we counted the number of leaves above the marked petiole.

Chlorophyll Assay

We used a spectrophotometric method for measuring chlorophyll concentrations. We ground 2 leaf discs from each plant in 200uL of magnesium carbonate (4MgCO$_3$Mg(OH)25H$_2$0) buffer. To extract the pigments, we then added 800uL of acetone. Light absorbence of each 1mL sample was then measured in a spectrophotometer at light wavelengths of 663nM, 645nM, 510nM, and 480nM. We then transformed these absorbencies into concentrations (ug/mL) by the following formulae:

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[Chl a](ug/mL) = 12.7E663 - 2.69E645 (MacKinney, 1941)
[Chl b](ug/mL) = 22.9E645 - 4.68E663 (MacKinney, 1941)
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To get concentrations in ug/cm^2, we used the following transformation:

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[Chl](ug/cm2) = [Chl](ug/mL) 1mL/2(pi)r2
              = [Chl](ug/mL) 1mL/2(pi)([(1/4in)/2]*2.54cm/in)2
              = [Chl](ug/mL) 1mL/0.633cm2
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Rubisco Assay

We measured Rubisco content in 3 plants from each treatment using an antibody assay. The antibodies were originally produced for Rubisco in *Chlamydomonas*; we used a Western blot to verify these antibodies also bind to Rubisco from *A. theophrasti*. To these primary antibodies we bound secondary antibodies to catalyze a color-change reaction. The density of this color is directly proportional to [Rubisco].

To quantify [Rubisco], we used a dot-blot technique. We placed an equal amount of each sample on an equal area of ordinary white paper. We blotted 4 separate dilutions for each plant. After binding the antibodies and producing the color-change reaction, we scanned the blot using a digitizing scanner. Then, using a computer program, determined the average darkness of each dot; this gave relative [Rubisco] for each of the 12 plants.

Results

Treatment Effectiveness

The data for relative growth rates is shown in Figure 1. Low-light plants grew more leaves than high-light plants. Low-nitrogen plants grew fewer leaves than high-nitrogen plants. This indicates the treatments did have some effect on plant growth and plants reallocate resources to light capturing in low light.

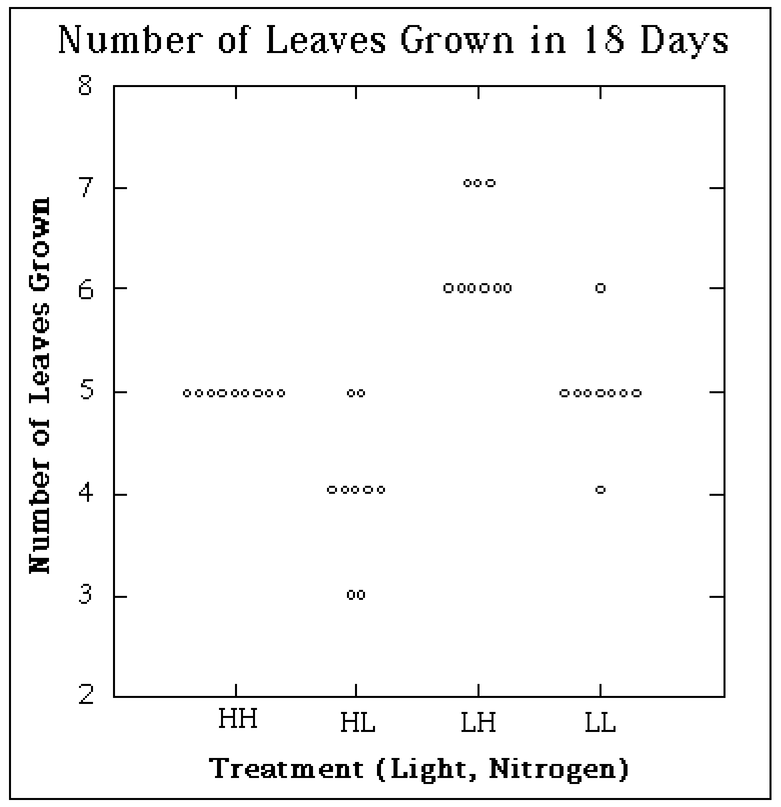

Figure 1. Relative growth of *Abutilon theophrasti* for each treatment. The ordered letter pairs, consisting of H (high) and L (low), designate the treatment type. The first letter represents the light treatment and the second letter represents the nitrogen treatment.

Chlorophyll Concentrations

Chlorophyll concentrations are shown in Figure 2 as a sum of both Chl a and Chl b. While low-light appears to have caused a slight decrease in [Chl], the variation within treatments is high enough to discount this small difference. The actual concentrations, however, agree with Besford (1986). He found tomato chlorophyll concentrations of 29-51 ug/cm^2.

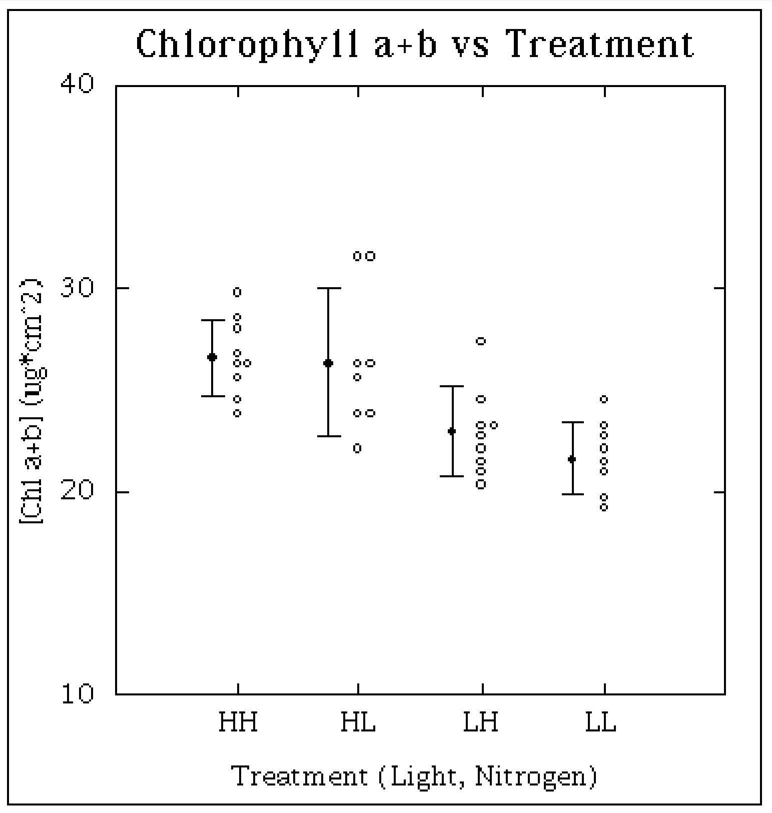

Figure 2. Chlorophyll concentrations for each treatment. The concentrations shown are the sum of Chl a and Chl b. Solid dots indicate means; bars are 2 standard deviations tall.

Rubisco Concentrations

Figure 3 shows Rubisco concentrations for 3 plants in each treatment. Since we did not have a standards lane of pure Rubisco, this graph indicates relative concentrations only. We were, however, able to determine from the dilutions that an increase of 11.5 on the left scale corresponds to a doubling in [Rubisco]. Again, the variation within treatments was as large as the variation between them.

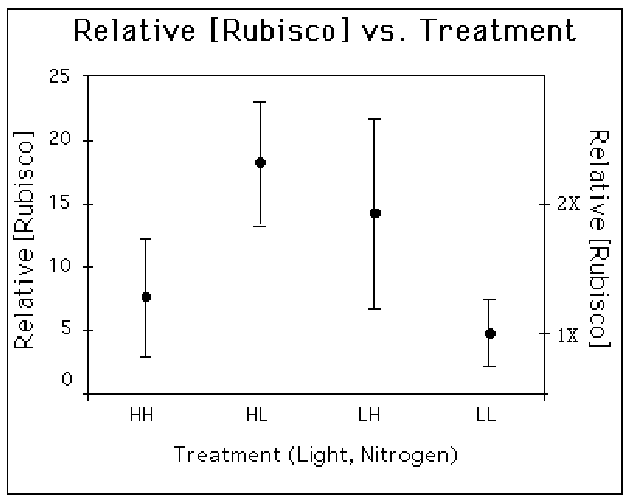

Figure 3. Rubisco concentrations for each treatment. The left vertical axis shows relative concentrations on an arbitrary scale; the right vertical axis indicates the distant required for a doubling of [Rubisco]. Solid dots indicate the mean; bars are 2 standard deviations tall. While we measured only 3 plants for each treatment, [Rubisco] calculations used good data from all 4 dilutions. Therefore, N=12 for HH, N=8 for HL, N=12 for LH, and N=11 for LL.

Conclusions

Though we found different rates of leaf production under high/low light and high/low nitrogen, we did not find differences in either chlorophyll or Rubisco concentrations. This does not support our hypothesis that plants partition, or balance, production of these two expensive molecules under these conditions. Nor can we state nitrogen-stressed plants have less Rubisco. This lack of support is due to the large amount of variation within treatments and the small amount of variation between them.

We suggest several reasons for the apparent lack of difference between treatments. First, plants may use some other method for controlling activity of these molecules. Rubisco is known to be controlled after translation (Ogren et. al., 1986). Rubisco has both an activase and a deactivase; plants likely control carbon-fixing with these enzymes rather than adjusting [Rubisco]. Second, the plants may not have actually been light or nitrogen stressed. Third, the small amount of data, particularly for [Rubisco], probably caused substantial sampling error.

Literature Cited

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